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Original Article

The Antiinfluenza Virus Activity of Hydroalcoholic Extract of Olive Leaves

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Abstract

The influenza viruses are major etiologic agents of human respiratory infections, and inflict a sizable health and economic burden. This study examines the antiinfluenza virus activity of hydroalcoholic extract of olive leaves (OLHE). Olive leaves were collected from gardens around the city of Shiraz, characterized, dried, ground to powder, and its hydroalcoholic extract was prepared. The influenza viruses were isolated from patients and characterized by standard antiinfluenza sera. Virucidal effects of OLHE (10^{-1} to 10^{3} µg/ml) were examined in pretreatment, treatment and incubation protocols using quantal assay after incubation for 72 h. All experiments were performed three times in quadruplicates. Pretreatment of the cell line with OLHE for one hour followed by the addition of the virus was associated with virucidal effects (1 to 1000 µg/ml). OLHE added one hour after incubation of the virus with cell did not show antiviral effects. OLHE incubated with the virus for one hour, and then added to the cell line did have antiviral activity (1 to 1000 µg/ml). The findings indicate that antiviral activity of OLHE occurred extracellularly, probably by changing the properties of membrane of the virus, rather than that of the cell, to prevent the virus from attaching and penetrating the cell line.

Keywords: Influenza virus; MDCK cell line; Olive leaf.
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1. Introduction

The olive tree (*Olea europea*) has been held high esteem throughout history. The winners in the Greek Olympic Games were crowned with a wreath of olive leaves, and in today's political literature a branch of olive is a symbol of peace and prosperity.

According to Iran's folk medicine, the olive leaves are of value to decrease blood pressure and glucose [1-3]. Experimental or clinical studies demonstrated that olive leaves have hypotensive [4, 5], vasodilatory, blood glucose lowering [6-8], as well as antioxidant and antiinflamatory [2, 3] activities. Moreover, the olive leaves have been reported to be active against malaria, fungi and bacteria [8-15].

Only two fairly recent reports have examined the antiviral activity of olive leaf extract [16, 17]. One of them [16] demonstrated that olive leaf extract inhibited

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Table 1. Virus yield induced by the vehicle and various concentrations of olive leaves extract (OLE) applied one hour before infection of the cell line with influenza virus.

OLE (μg/ml)	Virus yield (log 10-TCID50)
0.0	6.500 ± 0.000
0.1	6.166 ± 0.333
1.0	5.333 ± 0.083
10.0	4.833 ± 0.166*
100.0	3.166 ± 0.166*
1000.0	2.666 ± 0.440*

Data are presented as Mean±SEM. *Denotes significant ($p \leq 0.05$) difference from vehicle.

the replication of human immunodeficiency virus, and acute infection and cell to cell transmission of the virus. The other report [17] showed that olive leaf extract exhibited virucidal effect as indicated by decreasing the haemorrhagic septicemia rhabdovirus titer and protein accumulation.

The influenza viruses are major etiologic agents of human respiratory infections inflicting sizable health and economic burdens. To our knowledge, the effects of olive leaf extract on influenza virus have never been investigated. Herein, we report on the antiviral activity of hydroalcoholic extract of olive leaves.

2. Material and methods

2.1. Collection of olive leaves

Olea europea L. leaves were collected from local gardens around the city of Shiraz in November. The identity of the plant was characterized by an expert in the Department of Biology, College of Sciences, Shiraz University. A voucher specimen (40111) was kept in our laboratory for future references.

2.2. Preparation of hydroalcoholic extract

The leaves were dried in the shade, and ground to powder. Hundred grams of the powder was percolated with 1000 ml ethanol (70% v/v in water) for 72 hours. After evaporation of the hydroalcoholic extract over water bath, it was dried under vacuum [18]. The yield of extract was 38.6% (w/w).

2.3. Virus and cell line

The influenza virus was isolated from patients and characterized by standard antiinfluenza sera. The cell line (MDCK) was cultured in DMEM medium containing penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% heat-inactivated fetal bovine serum (GIBCO).

2.4. Determination of OLE cytotoxicity

Uninfected cells from MDCK cell line were grown in the absence and presence of various concentrations (10^{-1} to 10^{5} μg/ml) of OLE for 1, 2 and 4 days. Afterwards, the cells were exposed to trypan blue, loaded into haemocytometer, and the number of viable cells (unstained) was counted using low power of microscope. The number of cells per ml was then calculated [19].

2.5. OLE application and infectivity

Various concentrations of OLE, ranging from 10^{-1} to 10^{3} μg/ml, were prepared in medium containing 2 μg/ml trypsin and added to the cell monolayer 1 h prior to, 1 h after and simultaneous with 100 TCID50 of virus. In another series of experiments, appropriate concentrations of OLE and virus (100 TCID50) were incubated for one h in a refrigerator before addition to cell monolayers. Uninfected monolayer of cell line incubated with different concentrations of OLE was used as negative controls.

2.6. Infectivity assay

Virus titration was carried out in 96 microplates (NUNC), and was incubated for 3 days under 34 °C and 5% CO_2. The infectivity titer was determined by quantal

Table 2. Virus yield induced by the vehicle and various concentrations of olive leaves extract (OLE) applied one hour after infection of the cell line with influenza virus.

OLE (μg/ml)	Virus yield (log 10-TCID50)
0.0	6.167 ± 0.288
0.1	5.833 ± 0.288
1.0	5.500 ± 0.500
10.0	5.167 ± 0.288
100.0	5.000 ± 0.500
1000.0	4.667 ± 0.763

Data are presented as Mean±SEM.

assay [20]. All experiments were performed three times in quadruplicates.

2.7. Statistical analysis

Virus titers (mean SEM) were compared using One way analysis of variance (ANOVA). Where a significant difference was located with ANOVA, OLE-treated groups were compared with the control group using Dunnett test.

3. Results:

The results of cytotoxicity showed that the concentrations of 10^5 μg/ml and higher was toxic to MDCK cells. The results of application of various concentrations of OLE one hour before infection with influenza virus are shown in Table 1. The antiviral activity of OLE started at 10 μg/ml and increased dose-dependently reaching an order of magnitude of 4 at 1000 μg/ml. The results of application of various concentrations of OLE one hour after infection with influenza virus are shown in Table 2. The OLE did not produce significant inhibition of viral replication. Moreover, simultaneous application of various concentrations of OLE and the virus on the MDCK cell line showed no antiviral activity (data not shown).

The results of incubation for one hour at 4 °C of various concentrations of OLE and the virus are shown in Table 3. The statistically significant antiviral activity of OLE started at 1 μg/ml reaching a maximal effect (4 orders of magnitude) at 1000 μg/ml.

4. Discussion

The findings of the present study showed that the exposure of MDCK cells to OLE one hour before infection with influenza virus exhibited antiviral activities. It is, therefore, speculated that interaction of OLE with certain components of cellular membrane crucial to initiation of viral infection resulted in inhibition of viral entry into the cell.

On the other hand, incubation of the virus with OLE at 4 °C for one hour before adding to the cell also revealed antiviral activity. This might be due to the effect of OLE on the viral membrane thereby disabling the virus to initiate infection, by interference probably with viral attachment or adsorption. Similar profile was demonstrated with OLE, which exhibited antiviral activity against human Immuno-deficiency virus and haemorrhagic septicemia rhabdovirus [16, 17].

Table 3. Virus yield induced by the vehicle and various concentrations of olive leaves extract (OLE) incubated with the virus at 4 °C for one hour before addition to the cell line.

OLE (μg/ml)	Virus yield (log 10-TCID50)
0.0	6.500 ± 0.000
0.1	6.000 ± 0.288
1.0	4.910 ± 0.363*
10.0	4.330 ± 0.166*
100.0	3.000 ± 0.288*
1000.0	2.500 ± 0.000*

Data are presented as Mean±SEM. *Denotes significant ($p \leq 0.05$) difference from vehicle.

OLE was previously shown to interact with the phospholipid bilayers of cell surface [21]. Considering the similarities between viral and cellular membranes, the effect of OLE on lipid/protein components of Influenza virus envelope might account for antiviral activity, probably via blocking viral attachment or adsorption to the cell.

The mechanism of antiviral activity of OLE is unclear. However, OLE was shown to contain 3 major components, namely oleurepein, olenolic acid and hydroxyltyrosol. Moreover, pure eleuropein accounted for 85-90% of antiviral activity against human immunodeficiency virus [16]. Whether this antiviral activity was mainly due to oleuropein or other components remains to be investigated.

5. Conclusion

The findings of present study indicated that OLE exhibited activity against Influenza virus. Moreover, such antiviral effect might be due to the inhibition of viral adsorption or penetration. Further studies are needed to clarify the nature of its antivival effect.

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